ANA RITA PINHEIRO MARQUES

Design and prospective evaluation of a risk-based surveillance system and characterization of shrimp grow-out farms in northeast Brazil

Tese apresentada ao Programa de Pós-Graduação em Epidemiologia Experimental Aplicada às Zoonoses da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Doutor em Ciências

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São Paulo

2015

FACULDADE DE MEDICINA VETERINÁRIA E ZOOTECNIA

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que o Projeto intitulado "Desenvolvimento e avaliação prospectiva de um sistema de vigilância baseada em risco e caracterização de fazendas de engorda de camarão no nordeste do Brasil", protocolado sob o nº 2581/2012, não utilizando animais, sob a responsabilidade do Prof. Dr. Fernando Ferreira, está de acordo com os princípios éticos de experimentação animal da "Comissão de Ética no uso de animais" da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo e foi aprovado em reunião de 15/2/2012.

We certify that the Research "Design and prospective evaluation of a risk-based surveillance system and characterization of shrimp grow-out farms in northeast Brazil", protocol number 2581/2012, not using animals, under the responsibility Prof. Dr. Fernando Ferreira, agree with Ethical Principles in Animal Research adopted by "Ethic Committee in the use of animals" of the School of Veterinary Medicine and Animal Science of University of São Paulo and was approved in the meeting of day 2/15/2012.

São Paulo, 16 de novembro de 2015.

Denise Fantoni

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Aos amores da minha vida,

Mãe “Célinha” Maria do Céu e Papi “Chiquinho” Rui Marques.

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“É preciso ver o que não foi visto, ver outra vez o que se viu já, ver na Primavera o que se vira no Verão, ver de dia o que se viu de noite, com Sol onde primeiramente a chuva caía, ver a seara verde, o fruto maduro, a pedra que mudou de lugar, a sombra que aqui não estava.”

José Saramago

RESUMO

MARQUES, A. R. P. **Desenvolvimento e avaliação prospectiva de um sistema de vigilância baseada em risco para as fazendas de engorda de carcinicultura no nordeste do Brasil.** [**Design and prospective evaluation of a risk-based surveillance system and characterization of shrimp grow-out farms in northeast Brazil**]. 2015. 79 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2015.

O cultivo de camarão branco *Litopennaeus vannamei* tem provado ser um sector promissor para a economia do nordeste do Brasil. Contudo, a criação de camarão branco no Brasil tem sido afetada negativamente pela ocorrência de doenças virais, ameaçando a sua expansão e sustentabilidade. Por esta razão, depreende-se a importância da elaboração de um sistema de vigilância capaz de detectar e definir a ausência de doenças virais de elevado impacto econômico. O modelo estocástico AquaVigil é aqui implementado para avaliar prospectivamente diferentes estratégias de vigilância para determinar a ausência de doença e identificar a estratégia exigindo menor esforço de amostragem e simultaneamente, fazer o melhor uso dos recursos disponíveis através da implementação de vigilância baseada em risco. O estudo apresentado exemplifica a aplicação regional do sistema proposto para o estado do Ceará, podendo ser aplicado a outros estados do Brasil. O modelo AquaVigil pode analisar qualquer sistema de vigilância baseada em risco semelhante àquele aqui considerado. A criação de camarão no nordeste do Brasil tem sido alvo de vários desafios, desde a ocorrência de doenças virais a mudanças no acesso aos mercados internacionais. Tendo em consideração as dificuldades encontradas pela aquicultura de camarão no nordeste do Brasil, facilmente se compreende a importância de caracterizar e melhor compreender este setor e assim assegurar o seu desenvolvimento sustentável. Para este fim, foram aplicados métodos de análise de correspondência multipla e clustering particional a dados recolhidos durante um levantamento nacional de fazendas de carcinicultura de forma a obter informação necessária para caracterizar tendências e identificar falhas e necessidades existentes. Esta informação será útil no momento de melhorar o manejo das fazendas e elaborar legislação a favor do desenvolvimento do setor.

Palavras-chave: Sistema vigilância para camarão aquicultura. Carcinicultura Brasil. Vigilância baseada em risco. AquaVigil. Perfil de fazendas de carcinicultura nordeste Brasil. clustering hierárquico e particional.

ABSTRACT

MARQUES, A. R. P. **Design and prospective evaluation of a risk-based surveillance system and characterization of shrimp grow-out farms in northeast Brazil**. [**Desenvolvimento e avaliação prospectiva de um sistema de vigilância baseada em risco para as fazendas de engorda de carcinicultura no nordeste do Brasil**]. 2015. 79 p. Tese (Doutorado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2015.

The farming of Pacific white shrimp *Litopennaeus vannamei* in northeast Brazil, has proven to be a promising sector. However, the farming of Pacific white shrimp in Brazil has been affected negatively by the occurrence of viral diseases, threatening this sector's expansion and sustainability. For this reason, the drafting of a surveillance system for early detection and definition of freedom from viral diseases, whose occurrence could result in high economic loses, is of the utmost importance. The stochastic model AquaVigil was implemented to prospectively evaluate different surveillance strategies to determine freedom from disease and identify the strategy with the lowest sampling efforts, making the best use of available resources through risk-based surveillance. The worked example presented was designed for regional application for the state of Ceará and can easily be applied to other Brazilian states. The AquaVigil model can analyze any risk-based surveillance system that considers a similar outline to the strategy here presented. In recent years, shrimp aquaculture has faced many challenges, ranging from the occurrence of viral diseases to changes in market access. Considering the past and present challenges faced by the shrimp farmers in Northeast Brazil it is easily understood that the comprehensive characterization of the shrimp farming is of the utmost importance when striving for sustainable development. To this aim, the exploratory data analysis methods of multiple correspondence analysis and partitional clustering were applied to the data collected through a national census to extract the greatest amount of information and profile shrimp farms, identifying gaps and needs. The results of the analysis will contribute to improve management practices and policy-making for sustainable shrimp farming in Northeast Brazil.

Keywords: Surveillance system shrimp aquaculture. Shrimp aquaculture Brazil. Risk-based surveillance. AquaVigil. Profile shrimp aquaculture Northeast Brazil. Hierarchical clustering and partitioning.

SUMMARY

1 INTRODUCTION

1.1 Shrimp aquaculture in Brasil: present status

Shrimp farming in Brazil began in the 1970s in the northeastern region of the country, with the farming of Kuruma prawn *Penaeus japonicus*. The related difficulties in farming such species led to the alternative farming of domesticated native cultured species *Litopenaeus subtilis*, *Farfantepenaeus paulensis* and the Pacific white shrimp*Litopenaeus schimtti*. The aforementioned choice of farmed shrimp was also unsuccessful and a new chapter in Brazils shrimp farming began in the 1990s with the choice of *Litopenaeus vannamei* as the main cultured species. Viable production was then observed with special reference to the years of 1995 to 1996, and the shrimp farming sector has expanded ever since (MAGALHÃES, 2004; COSTA, 2010). The Pacific white shrimp proved to be advantageous due to its high growth rate, high stocking density, tolerance to a wide interval of salinities and temperatures and high survival rates in shrimp hatcheries (BRIGGS et al., 2004; COSTA, 2010). To date, the Pacific white shrimp is the only cultured shrimp species in Brazil.

Shrimp grow-out farms and hatcheries are spread along Brazil's extended coastline and river-systems in the northeast states of Pará, Maranhão, Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe and Bahia and to the south, the states of Rio Grande do Sul, Santa Catarina and Paraná (ABCC/MPA, 2013). There is potential for further expansion and shrimp farming is regarded as a valuable sector.

Census data for 2011 revealed that shrimp farming in Brazil produced a total of 69571 tons of shrimp for a cultivating area of 19845 ha. Previous census data for 2004 indicated a higher production of 75904 tons and cultivated area of 16598 ha (ABCC/MPA, 2013).The decreased levels of production can be due to factors such as the anti-dumping measures from the United States of America, one of the major exporting countries for Brazil's cultured shrimp, along with the depreciation of the Brazilian BRL against the US Dollar and the decrease in cultivated shrimp densities, this final factor possibly related to the occurrence of viral diseases (DE ABREU et al., 2011; ABCC/MPA, 2013). The aforementioned anti-dumping policy and BRL depreciation dictated a loss of competitivity for international trade and the shift of market access towards the national market (ABCC/MPA, 2013). The detrimental factor in the survival of Brazilian shrimp farming was the very increase in internal consumption. Around 98% of the shrimp produced in Brazil are destined for national consumption, evident in the increase from 270 g *per capita* in 2005 to 500 g *per capita* in 2009. Presently, the internal market presents potential to absorb the shrimp farmed in Brazil, with a foreseeable need to export farmed shrimp to foreign markets (ROCHA, 2011).

The sustainability of shrimp farming in Brazil was threatened by the occurrence of disease outbreaks of Infectious Myonecrosis in 2004 in the Northeastern states and to the south, by outbreaks of White Spot Syndrome in 2005. Productivity was seriously diminished and farmers questioned the viability of future shrimp farming (DA SILVA et al., 2010). The shrimp sector survived by adopting better management practices. These framers recognize the threat of viral diseases to sustainable production, especially those of viral etiology, having decreased production density and strengthened control of the sedimented organic matter of earthen ponds (DE ABREU et al., 2011).

1.1.1 Shrimp farming in northeast Brazil

In northeast Brazil, shrimp farming averages 3 yearly cycles with cultivated areas of 19610 ha and 69171 tons of shrimp produced for year 2011 (ABCC/MPA, 2013). Brazil's northeastern states account for 99,3% of the total of shrimp produced in Brazil for 2011. In this region, shrimp farming is an important source of revenue for commercial farmers, with particular importance for small-scale rural farmers, for whom shrimp farming is a supplementary or main source of income and poverty alleviation (ABCC/MPA, 2013). In this region, 59% of farms are small-scale, with less than 5 ha of farmed area, highlighting the important contribution of the small-scale farmers in Brazilian aquaculture (ABCC/MPA, 2013). The vulnerability of shrimp farming in the northeastern states of Brazil is apparent as it is mainly comprised of farmers with low investment capability (ABCC/MPA, 2013). These lower-income farmers face greater challenges when dealing with the threat of disease occurrence and outbreaks. The global demand and consequent increased production of *L. vannamei* can further threaten the small-scale farmer's livelihoods and competitivity with more efficient, intense and well equipped farming systems, producing more affordable and better quality shrimp in environmental friendly farming systems (FAO, 2013).

When considering the past and present challenges, the sustainability of shrimp farming in Brazil depends on the increased technification of the production systems, better jurisdiction and environmental management and general support for shrimp farmers (DE ABREU et al., 2011).

1.2 Aquatic animal health and sustainable production

The production of aquatic animals through aquaculture is presently the worlds most rapidly expanding food-producing sector, greatly due to the growing demand for aquatic animals and their products and the low response capacity of commercial extractive fishing. As a result, aquaculture activities have evolved and intensified greatly (SUBASINGHE; MCGLADDERY; HILL, 2004).

Not only will the global trend towards the increased consumption of aquacultured crustaceans and their products increase pressure on aquaculture and the extractive fisheries sector, so will the threat of viral diseases affecting crustacean populations worldwide seriously compromise sustainable production and increase the chances of fishermen and farmers abandoning such activities. Low technification and awareness can further increase the damage brought on by the aforementioned factors. The present burden of increased impositions and constraints on the trade of aquatic animals and aquatic animal products is a testimony of the threat to the aquaculture sectors sustainability (STENTIFORD et al., 2012).

1.2.1 The role of aquatic animal health surveillance

The Agreement on the Application of Sanitary and Phytosanitary Measures (SPS Agreement) drafted by the World Trade Organization (WTO) recognizes the need for its member countries to adopt measures to protect their aquatic animal populations by managing the risk associated with imported trade commodities, without creating unjustified trade barriers. Importing countries should determine what is their appropriate level of protection (ALOP) and request trade partners to achieve a level of equivalency in order to facilitate trade. This level of protection should be in accordance with the importing country's disease status and surveillance efforts to determine and maintain disease-free status (OIE, 2012a). Among measures to protect the health of a country's aquatic animal population are import risk analysis (IRA) of aquatic animals and aquatic animal products. IRA relies on the availability of valid scientific evidence generated from well-controlled ongoing surveillance for an importing country to be able to justify disease-free status, apply trade restrictions, request quarantining, among other measures (SUBASINGHE; MCGLADDERY; HILL, 2004).

The World Organization for Animal Health (OIE) and the the Food and Agriculture Organization of the United Nations (FAO) have elaborated standards and guidelines to maintain and respond to the threat of viral diseases to aquacultured and wild aquatic animals. Countries can adopt the aforementioned as the guiding principals to apply to promote safe international trade. Among the available documentation special reference can be made to the manuals for *"Understanding and applying risk analysis in aquaculture: a manual for decision makers"* and *"Surveillance and zoning for aquatic animal diseases-Technical paper 451"* (SUBASINGHE; MCGLADDERY; HILL, 2004; ARTHUR et al., 2009). The OIE in particular drafted a strategic plan for 2011-2015 highlighting the need for healthy production of aquatic animals by supporting member countries in strengthening their veterinary services through the *"OIE World Animal Health and Welfare Fund"* (OIE, 2004). Specific OIE documentation for shrimp health management and surveillance is available through publications such as the *"Manual of Diagnostic Tests for Aquatic Animals"* and the *"OIE Aquatic Animal Health Code"* (OIE, 2012a, 2012b). Implementing aquatic animal surveillance can help to define and maintain disease-free status for a country or zone, provide knowledge of disease occurrence for control and eradication of specific pathogens and inform trade partners of OIE listed notifiable diseases, promoting safe production and safe international trade (OIE, 2012a).

1.3 Aquatic animal health in Brazil

The intensification of aquaculture production has resulted in increased trade and translocation of aquatic animals and aquatic animal products. Consequently, we are witnessing the spread of diseases affecting aquatic animals on a larger, global scale. The growing threat of the spread of diseases affecting shrimp farming and extractive fishing of crustaceans leads to the increased demand for products with low risk of disease from countries able to supply them (STENTIFORD et al., 2012). The shrimp farming in Brazil is no exception to the global trend of aquaculture in terms of intensification, increased trade and spread of disease. The inherent importance of developing strategies to safeguard the health of the shrimp population, nationally and regionally, should focus on efforts for timely diagnosis, monitoring and reporting of disease occurrence (STENTIFORD et al., 2012). Brazilian authorities recognize the importance of safeguarding the health of aquatic animals. Therefore, the Brazilian Ministry of Fisheries and Aquaculture (MPA) has adopted a set of measures, among them IRA to assess the risk associated with the importation of aquatic animals and their products, as these may pose a potential threat to the health of wild and aquacultured species in the country. In an attempt to mitigate such risks, Brazil legislated a series of measures including, most importantly, a set of normative instructions and ordinances. The Normative Instruction 39, of November 4, 1999 was developed in response to serious outbreaks of disease affecting shrimp farming worldwide. This normative instruction determined the suspension of the entry into the country of all species of aquatic animals, either from fresh or salt water, at any stage of their life cycle, including their fresh and frozen products, as well as cooked when in their shells or parts of them, from any source. Furthermore, this normative instruction conditioned the authorization of imports to be anticipated by IRA by the agropecuary defense secretariat's Department of Animal Health, which would take into account the animal health situation in the countries of origin (BRASIL, 1999). A second normative instruction recognizing the need to implement health management measures in aquatic animal populations was implemented on July, 2003, approving the "National Program for Aquatic Animal Health". It considered the need to standardize prophylactic actions, diagnosis and sanitation of aquaculture establishments and defined the role of public organizations for animal health protection in the fight against diseases affecting aquatic animals. Furthermore, this normative instruction approved the "Technical Regulations of the National Program of Health of Aquatic Animals" (BRASIL, 2003). Three separate legislative measures were drafted appointing responsibilities to the recently extinct MPA and that have been passed on to the Ministry of Agriculture (MAPA). Among them are the normative instruction instituting the National Network of Laboratories of the MPA (RENAQUA), responsible for official diagnostics of diseases in aquatic animals and research in aquatic animal diagnostics (BRASIL, 2012). The normative instruction no.14 of 9 de December, 2010 drafted the general procedures for IRA for aquatic animals and aquatic animal products (BRASIL, 2010). Lastly, the MPA ministerial ordinance deliberated the implementation of the "Interministerial Committee for the Defense of Aquatic Animals" to supervise activities related with the preservation of aquatic animal health (BRASIL, 2015a). To secure the feasibility of the majority of the objectives of the aforementioned normative instructions and ordinances, information on the occurrence and distribution of diseases affecting farmed and wild species needs to be generated by a national surveillance system. A structured surveillance system for crustacean species, based on regular sample collecting, laboratory diagnosis and disease reporting, has yet to be implemented in Brazil. The surveillance system should be dratted taking into consideration OIE guidelines and be able to satisfy the following provisions (SUBASINGHE; MCGLADDERY; HILL, 2004):

- generate information to demonstrate the presence or absence of infection in farms, zones or at the country level, to assure the safe translocation of aquatic animals and their products at the national and international level;

- support early disease detection for notifiable diseases;

- provide information to determine the effectiveness of disease control or eradication programs.

1.3.1 Notifiable diseases for aquatic animal surveillance

The OIE drafts and periodically revises a list of notifiable diseases for aquatic animals. The choice of diseases is based on the infectious agents capacity to disseminate, the consequences to production and overall impact on cultured and wild aquatic animals and on the existence of a repeatable and robust means of detection/diagnosis (OIE, 2012a). The MPA drafted a list of notifiable diseases in crustacean species for compulsory notification (BRASIL, 2015b).

- Yellow head disease (YHD);

- Infectious haematopoietic necrosis (IHN);

- Infectious myonecrosis (IMN);

- Necrotising hepatopancreatitis (NHP);

- Taura syndrome (TS);

- White spot disease (WSD);

- White tail disease (WTD);

- Early mortality syndrome (EMS);

- Baculoviral midgut-gland necrosis (BMN);

- Hepatopancreatic parvovirus disease (HPV);

- Mourilyan virus infection (MVD);

- Spawner-isolated mortality virus disease (SMV);

- Penaeus monodon-type baculovirus (BMV);

- Tetrahedral baculovirosis (TBP).

The listed diseases resemble the notifiable diseases listed by the OIE with the inclusion of early mortality syndrome (EMS), baculoviral midgut-gland necrosis (BMN), hepatopancreatic parvovirus disease (HPV), mourilyan virus infection (MVD), spawner-isolated mortality virus disease (SMV), *Penaeus monodon*-type baculovirus (BMV) and tetrahedral baculovirosis (TBP) .The MPA officially recognizes the presence of WSD (2005, 2010 and 2011), IMN (2008 and 2009) and IHN (2009 and 2010) (MPA, 2014). The needed surveillance system should be drafted considering these listed notifiable diseases affecting crustacean species.

1.4 Surveillance and characterization of shrimp farming in Brazil

The importance of implementing a surveillance system in Brazil is highlighted from the aforementioned topics. The prospective evaluation of any future surveillance system for important pathogens affecting shrimp farming is of the utmost importance to determine the necessary efforts to successfully implement aquatic animal surveillance in Brazil. The surveillance approach should also be based on the characterization of shrimp grow-out farms by risk of introduction of important listed pathogens. A risk-based approach to surveillance minimizes the number of sampled farms and animals needed to establish disease-free status, making best use of available resources, for viable surveillance system implementation. The methodology and necessary tools to prospectively evaluate such surveillance efforts is proposed in Chapter 1 of the present thesis.

Furthermore, sustainable development can only be achieved after performing a thorough characterization of the present trends in shrimp aquaculturing in the major shrimp producing region of northeast Brazil. In Chapter 2 of the present thesis, the characterization of the shrimp grow-out farms in northeast Brazil is achieved by means of a data denoising method of multiple correspondence analysis and present trends analyzed after performing hierarchical clustering on the resulting data.

Design and prospective evaluation of a risk-based surveillance system for shrimp grow-out farms in northeast Brazil

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Abstract

The farming of Pacific white shrimp *Litopennaeus vannamei* in northeast Brazil, has proven to be a promising sector. However, the farming of Pacific white shrimp in Brazil has been affected negatively by the occurrence of viral diseases, threatening this sector's expansion and sustainability. For this reason, the drafting of a surveillance system for early detection and definition of freedom from viral diseases, whose occurrence could result in high economic loses, is of the utmost importance. The stochastic model AquaVigil was implemented to prospectively evaluate different surveillance strategies to determine freedom from disease and identify the strategy with the lowest sampling efforts, making the best use of available resources through risk-based surveillance. The worked example presented was designed for regional application for the state of Ceará and can easily be applied to other Brazilian states. The AquaVigil model can analyse any risk-based surveillance system that considers a similar outline to the strategy here presented.

Keywords: Surveillance system shrimp aquaculture; Shrimp aquaculture Brazil; Risk-based surveillance; AquaVigil.

1. Introduction

The growing global demand of aquatic animals and aquatic animal products has led to high rates of production and trade frequency and increased concern over the occurrence and spread of viral diseases affecting various cultured species. As a result, many nations have adopted surveillance strategies to protect their aquaculture sector (FAO, 2014). The threat to aquaculture sustainability and safe international trade has also led countries to apply trade standards based on their own aquatic animal health status (FAO, 2014). When able to demonstrate that a particular disease agent is absent, a country can facilitate trade or apply import risk analysis (FAO, 2014; WTO, 2014).

The absence of infection, from here on referred to as freedom from disease, can be determined through the aggregation over time of negative outcomes generated from a surveillance system. Documenting freedom from disease requires a large sample frame and so surveillance activities should selectively target the high-risk strata of the population through risk-based surveillance (RBS) (Cameron, 2009). In this paper, a stochastic model based on scenario tree modelling was implemented to determine the probability of freedom obtained for certain surveillance efforts, so that the best strategy can be determined prospectively. The model outputs will provide decision makers with the information to implement surveillance efforts to achieve a desired probability of freedom. The surveillance system will also ensure early disease detection.

In northeast Brazil, the farming of Pacific white shrimp *Litopennaeus vannamei* has been an important source of income for large-scale producers and also the main or supplementary income-generating activity for the poorest rural communities and their small-scale farmers. In many cases, the occurrence of viral diseases has lead to the abandonment of farming activities (Ostrensky et al., 2008). There is to date, unclear knowledge on the geographic extent and impact to which viral diseases have affected the countries shrimp aquaculture sector. From the list of notifiable viral diseases in shrimp populations drafted by the Brazilian Ministry of Fisheries and Aquaculture (MPA), the Centre for Environment, Fisheries and Aquaculture Science (Cefas) references the presence of White Spot Syndrome Virus (WSSV), Infectious Myonecrosis Virus (IMNV), Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) and Taura Syndrome Virus (TSV), initially ruling out efforts for determining country-level freedom. Meanwhile, other viral pathogens such as Yellowhead Virus (YV) have yet to be identified at a national level (CEFAS, 2014; DOU, 2015).

The MPA has recognized the threat of viral diseases to the aquacultured shrimp populations and the need to strengthen disease surveillance (MPA, 2011). The needed surveillance system must encounter international acceptance and therefore follow World Organisation for Animal Health (OIE) surveillance guidelines (Corsin et al., 2009; OIE, 2014a). Implementing surveillance efforts to declare disease-free status can lead to early disease detection and identification of disease-free zones. The model can be applied to any one of the listed notifiable viral diseases, as the epidemiology, pathogenicity and many clinical features are similar among them.

2. Methods

2.1. Model overview

The AquaVigil model evaluates the results of implementing two types of surveillance system components that are activities that generate the needed information to determine freedom from disease: one active surveillance system component (ActiveSC) and one passive surveillance system component (PassiveSC). Surveillance system sensitivity (SSe), that is, the probability of the surveillance system detecting disease if it were present, can be estimated considering the joint contribution of the two surveillance components that make up the surveillance system: the PassiveSC sensitivity (SePassiveSC) and the ActiveSC sensitivity (SeActiveSC).

The model was developed in R environment and is available as the AquaVigil function in the Supplementary Document 1 with example data in the Supplementary Document 2 and using packages mc2d, plyr, ggplot and Hmisc (R Development Core Team, 2008; R CRAN, 2015). The simulation comprised of 10000 iterations and set a fixed random number seed for reproducible random results. Model inputs necessary for analysis are a comma-separated values (CSV) file with an ID column, four columns characterizing the presence (1) or absence (0) of four risk factors (RFs) and a fifth column specifying the number of samples retrieved from each farm. Prospectively, we can determine the SePassiveSC, the SeActiveSC, the SSe, the probability of freedom obtained through surveillance, the sample size for the ActiveSC and campaigns needed to achieve a desired probability of freedom. Other model outputs include a correlation analysis for surveillance system component sensitivities, the achieved probability of freedom after a single surveillance campaign and the sensitivity ratio for the ActiveSC (SR).

2.1.1. Data sources

A past census of the productive, technological, economical, social and environmental aspects of Brazil's aquaculture sector was drafted for the year of 2011 and the data provided by shrimp grow-out farms when questioned for this census was the data here used. From the available data, the worked example presented for determining disease freedom for the state of Ceará accounts for 325 grow-out farms, parameterized for the presence and absence of the selected RFs. The census data provided the coordinates for 273 of the 325 farms. To roughly illustrate the density of farmed areas, a map of such farms is provided in Fig. 1. From this map, the main farming areas are visible along the Jaguaribe River and delta, to the South, and the Acaraú River delta, to the North (ABCC/MPA, 2013).

2.1.2. States to define disease-free zones

Dispersion of infectious agents through water occurs frequently and at a rapid rate (Hoa et al., 2011; Lotz, 1997; Moss et al., 2012). This would strongly suggest the rapid spread of the pathogens between farms in interconnecting water systems. Therefore, the level at which disease freedom can be defined should be reasonably large. Furthermore, shrimp grow-out farms will frequently use post-larvae (PL) from PL suppliers in their state. Given the previous considerations, the state level was considered to define disease freedom. The state level will also allow a more efficient organizational approach to surveillance. A worked example of the AquaVigil model for Brazil's state of Ceará is presented, as this was determined as one of the leading states for aquacultured shrimp production (ABCC/MPA, 2013).

2.1.3. Surveillance of farmed populations

The "Report of the meeting of the Task Force on Animal Disease Surveillance Brussels, 24 and 25 June 2009" addressed how to demonstrate disease freedom from WSSV. The task force discussed how surveillance at the farm level would suffice when the disease is well known and the population in the farms is representative of both wild and farmed populations (European Commission, 2009). Taking into account the aforementioned conditions and the data available, only sampling of shrimp from grow-out farms was considered.

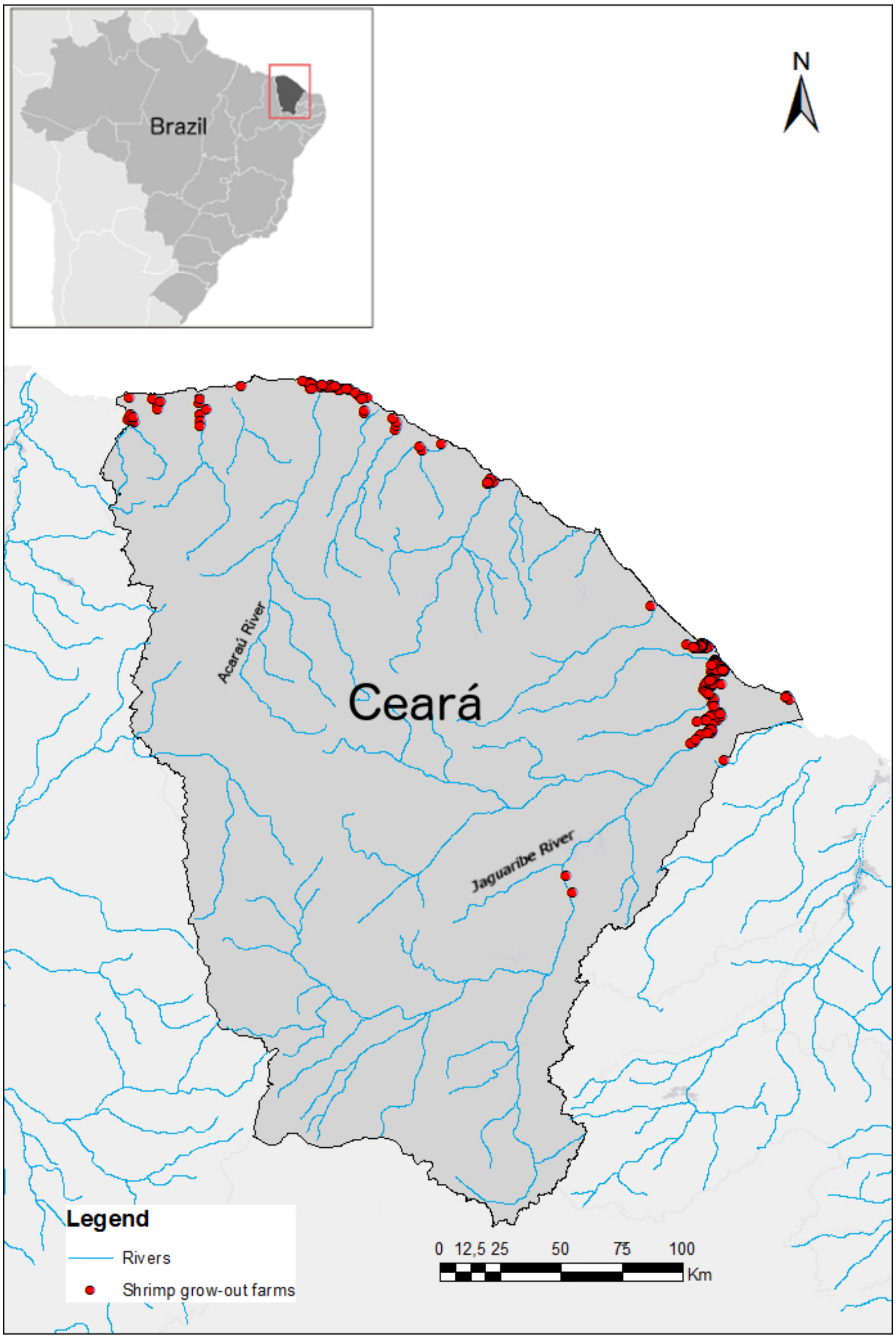

Figure 1: Map of shrimp grow-out farms in the state of Ceará.

2.1.4. Time period for analysis

Australian authorities considered one campaign as sufficient to determine disease freedom for WSSV (East et al., 2004; East et al., 2005). Here we consider a single campaign both sufficient and most desirable to determine a cost-effective surveillance strategy, given the contribution of both surveillance system components. The water temperatures that could determine a higher probability of clinical signs of disease at certain time periods, are relatively constant for Brazil's northeast states, and so there is no time of year recommended to perform surveillance activities (Nunes et al., 2005). Therefore, the time periods for analysis of the surveillance system results will cover one year of surveillance.

2.1.5. Design prevalence

In the absence of positive findings, the surveillance system can be applied to determine that disease is absent at a level equal to or greater than that of the design prevalence (Cameron, 2009). To incorporate the effect of *clustering*, two values of design prevalence can be used in the model: animal level design prevalence (Pu*) and farm level design prevalence (Ph*). For highly contagious diseases, such as those affecting species in an aquatic environment, a high proportion of infected animals are expected, and therefore, high values of design prevalence can be used (Martin et al., 2007). The OIE recommends the adoption of values of 1% to 5% for Pu* for slow-moving diseases and above 5% for highly contagious diseases, while values for Ph* should not exceed 2%, unless clearly justified (Corsin et al., 2009). However, published articles of Australian surveys, to determine country-level freedom from WSSV, used two sets of values for Ph* and Pu*: one where both took the fixed value of 10% and another where Ph* was set at 5% and Pu* was set at 10% (East et al., 2004; East et al., 2005). Keeping in mind the aforementioned values, for the worked example, the design prevalence was set at a value of 10% for Pu*, while the value of Ph* was kept conservatively lower, at 5%.

2.1.6. Test sensitivity

The OIE Manual of Diagnostic Tests for Aquatic Animals refers to molecular techniques, such as PCR followed by sequencing, for targeted surveillance to declare freedom for viral diseases affecting shrimp (Corsin et al., 2009; OIE, 2014b). The test sensitivity, a measure of the tests ability to identify truly infected animals, is considered to be high for tests based on molecular techniques (Corsin et al., 2009). A survey to determine disease-free status for WSSV in Australia considered a point value of 95% test sensitivity for the OIE recommended PCR protocol from Lo et al., (1996) (East et al., 2005; OIE, 2014b). Consequently, these were the values considered for the test sensitivity for both initial PCR screening and confirmatory diagnosis through sequencing. The combined test sensitivity was set as the product of individual test sensitivities. Since follow-up testing to investigate true status of infection is always applied, the specificity of the testing protocol is considered to be 100%.

2.2. Active SSC

Active surveillance generates information on the health status of a population through the periodic collection of samples. In order to reduce the sample size needed to determine disease-free status, the ActiveSC will account for targeted sampling of grow-out farms at greatest risk of disease introduction, through RBS.

2.2.1. Scenario tree

A scenario tree illustrates the process by which the surveillance component can result in disease detection. In the scenario tree, factors affecting the probability that an individual unit or grouping level of units is infected are taken into account to determine the SeActiveSC. The SeActiveSC was determined using the methodology of Martin et al.,(2007) based on the conceptual scenario tree in Fig. 2. For reasons of practicality, the scenario tree illustrates but one possible path to disease detection, considering high-risk farms those accounting for the presence of all RFs. Furthermore, the RFs are considered independent and their order unimportant in the scenario tree.

2.2.2. SeActiveSC

The relative risks (RRs) for the RFs represented as risk nodes in the scenario tree are adjusted according to Eq.(1) and Eq.(2) so that the average RR of a representative sample of the reference population in a risk node is 1, while maintaining a relativity specified in the inputs.

$$AR_1 = RR_1/(RR_1 * PropRR_1 + PropRR_0) \quad (1)$$

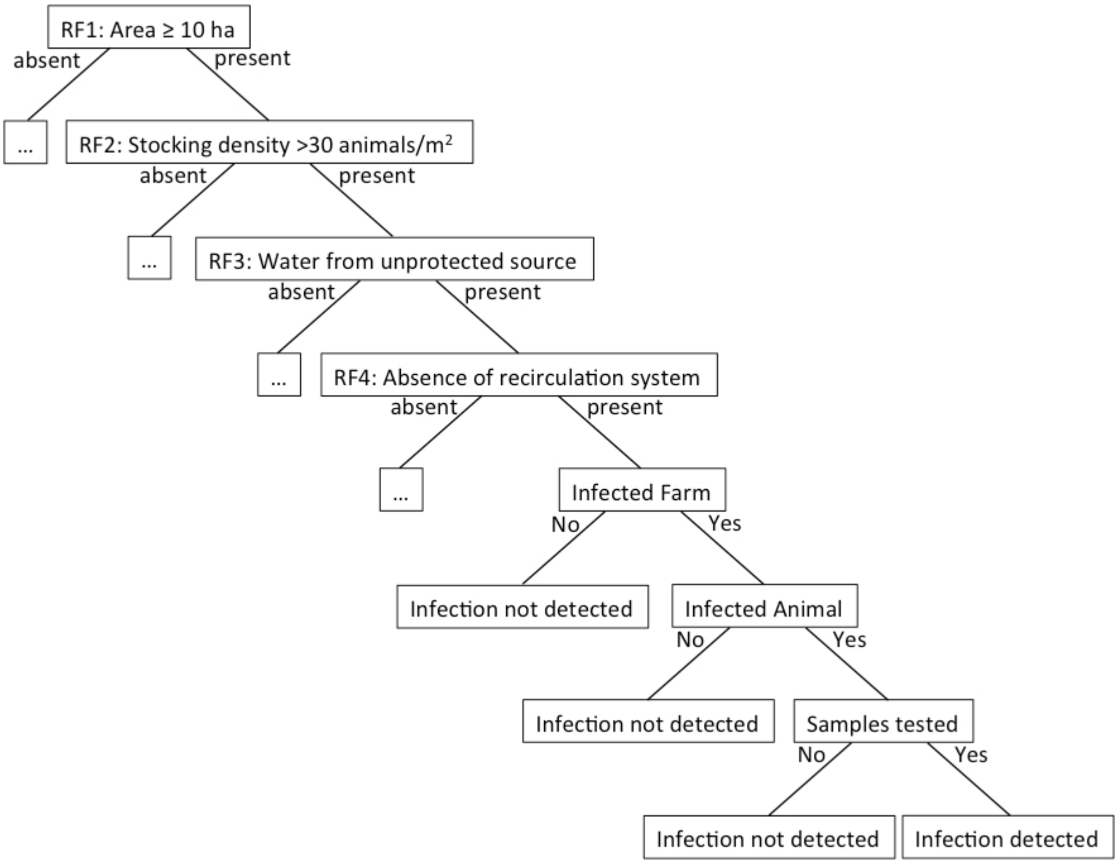

Figure 2: The scenario tree for the active surveillance system component.

$$AR_0 = 1/(RR_1 * PropRR_1 + PropRR_0) \tag{2}$$

where AR_1 and AR_0 are the adjusted risks for the RRs presence (1) and absence (0) and $PropRR_1$ and $PropRR_0$ are the proportions of the reference population that fall into the two branches of the risk node. The AR values are used to determine the effective probability of a farm being infected (EPIH), if infection is present at Ph^* (Eq. 3).

$$EPIH_s = Ph^* * \prod_{r=1}^{R}(AR_r) \tag{3}$$

where R is the number of RFs and $EPIH_s$ is determined for every s risk strata. The AquaVigil model accounts for four RFs, each with two possible outcomes, for a total of 16 ($=2^4$) risk strata. The unit sensitivity (SeU) is the probability that a single animal will give a positive result, if tested, and is set as the product of initial and confirmatory test sensitivities. When SeU is multiplied by the probability of a unit being infected, Pu^*, we have the probability of a positive test result for a single randomly selected unit. The grow-out farm level sensitivity (SeH) is the probability that infection will be detected at the farm level. For each sampled farm, a value of SeH is determined by the binomial method (Eq. 4), as the number of shrimp sampled per farm will be lower than 10% of the total number of shrimp in a grow-out farm.

$$SeH = 1 - (1 - SeU * Pu^*)^n \tag{4}$$

where n is the number of animals sampled for each farm.

To determine the SeActiveSC, the probability of detecting infection at the state grouping level, the hypergeometric method is used since the number of sampled farms will be above 10% of the total number of farms in the state (Eq.5).

$$SeActiveSC = 1 - \prod_{s=1}^{S} (1 - SeHav_s \, * \, n_s/N_s)^{(N_s * EPIH_s)} \tag{5}$$

for s risk strata, where:

- SeHav_s is the average SeH for the sth risk strata;
- n_s is the number of sampled farms in the sth risk strata;
- N_s is the number of farms in the population for the sth risk strata.

2.2.3. Sensitivity ratio

In order to compare the sensitivity of targeted risk-based sampling with equivalent random sampling, a SR of these values is returned as an output of the AquaVigil model to assess the effectiveness of the targeted surveillance approach. An equivalent random sample was considered a sample with the same number of farms and the same number of sampled animals per farm. A SR superior to 1 indicates a gain in sensitivity, while lower values reflect loss in sensitivity and values equal to one an equal sensitivity between sampling strategies.

2.2.4. Random sampling

The samples collected for RBS are compared to the needed samples size if performing representative random sampling of the population. The number of sampled farms ($N_{representative}$) (Eq. 6) and animals ($n_{representative}$) (Eq. 7) per farm for a representative random sample was determined using a two-stage sampling method (Cameron et al., 2014).

$$N_{representative} = (N/SeH_{desired}) * (1 - (1 - SSe_{desired})^{(1/(Ph^{*} * N))}) \tag{6}$$

$$n_{representative} = ln(1 - SeH_{desired})/ln(1 - SeU * Pu^{*}) \tag{7}$$

where:

- N is the number of farms in the state;
- $SeH_{desired}$ is the desired farm level sensitivity;
- $SSe_{desired}$ is the desired sensitivity for the surveillance system;
- Ph* is the design prevalence at farm level;
- SeU is the product of diagnostic test sensitivities;
- Pu* is the design prevalence at the animal level.

A desired system sensitivity of 95% and farm level sensitivity of 95% were considered, as these would be values commonly used for two-stage sampling to determining disease-free status in a single sampling campaign.

2.2.5. RFs for introduction of infection at the farm-level

Sampling high-risk farms aids early disease detection and lowers sampling efforts for surveillance. Scientifically documented RFs for introduction of shrimp viral diseases at grow-out farm level need to be identified to perform targeted sampling of the high-risk farms. However, in the absence of specific publications, RFs were selected from the data collected in the census data for 2011. The authors considered four RFs to be a reasonable number to allow the differentiation of a sufficient number of high-risk farms. The high-risk grow-out farms were considered those where all four RFs for the introduction of infection were present.

Farming intensities. When looking to enhance disease detection, one should consider the intensity of farming practices. Frequently, the intensity of farming can be inferred from both the size of the farms and the density of shrimp cultivated in the grow-out ponds. Larger farms are more likely to use large volumes of non-treated water, receive numerous shipments of PL to stock the farms and are subject to an increased movement of people, vehicles and animals, which can serve as pathways for disease introduction (Lightner, 2005; Lotz, 1997). Therefore, the RF characterizing the type of grow-out farm in terms of production was considered. This RF was present for large to medium scale grow-out farms, as the RF "cultivating areas equal to or above 10 hectares", absent for micro and small producers, with lower cultivating areas.
Shrimp grow-out farms also differ in the densities of shrimp cultivated per square meter. Farms in Ceará apply varying stocking densities, irrespectively of farm size, increasing intensity of shipments of PL onto the farm and increasing the risk of disease introduction. Furthermore, applying high stocking densities to production systems without the proper management can lead to increased stressing of the shrimp and risk of disease occurrence (Kautsky et al., 2000). A second RF indicating the high density of shrimp is therefore considered present as the RF "densities above 30 or more shrimp per square meter" are stocked, absent when stocking densities are equal to or below this value. The values for the aforementioned RFs were chosen as they are used in Brazil to distinguish small and medium-scale farms (ABCC/MPA, 2013).

Biosecurity. Biosecurity measures are implemented at the aquaculture establishment to reduce the likelihood of introduction of infectious pathogens (Lotz, 1997). Therefore, biosecurity measures are important when determining which RFs to include in the model. Pathogen exclusion is frequently accomplished by stocking farms with controlled water sources and disease-free shrimp (Lightner, 2005). The importance of stocking farms with PL free from disease, either certified through rigorous testing or supplied as specific pathogen free PL (SPF) is central to biosecurity in shrimp aquaculture (Bray et al., 2004; Clifford and Cook, 2002; Corsin et al., 2005; Hoa, et al., 2005; Lightner, 2003; Lightner, 2005; Lotz, 1997; Walker et al., 2011). However, information on the use of SPF PL was not available from the census that supplied the data for analysis. On the other hand, the census did provide information on the main source of water supplied to the farm. The water used for shrimp farming is considered as one of the most significant ways of pathogens introduction at the farm level (Moss et al., 2012). A third RF was therefore considered, the use of "water from unprotected source", where water supplied from a source other than a well is regarded as a source of pathogen introduction. Limiting pathogen introduction can also be accomplished through "zero" water exchange (Lightner, 2005). A biosecure grow-out farm will limit water exchange by means of a recirculating (Lotz, 1997). Consequently, a fourth RF was considered, the "absence of a recirculation system". Since there is no true "zero" water exchange and water can be supplied from an unprotected source, these RFs are not considered dependent nor redundant. Once more, the census provided information on the water source and use of a recirculation system (ABCC/MPA, 2013).

2.2.6. Relative risks

The accuracy of the model depends heavily on the values chosen for this key model parameter. This is among one of the recognizable difficulties that currently limit the developing of RBS in the aquatic context. A common approach to estimating such parameters is the use of information from expert panels. Experts in the field of shrimp health in Brazil that could provide adequate estimates are scarce. Furthermore, the reliability of any estimates that could be provided would be greatly uncertain, given the present knowledge of occurrence of shrimp diseases in Brazil. The still-developing field of aquatic animal surveillance and specific epidemiological studies also made literary references to support the choice of values unattainable (Oidtmann et al., 2013; Peeler et al. 2011).

Given the previous considerations, values for RR were assigned arbitrarily in a conservative way that would reflect the underestimation of the importance of any RF. The RFs were equally weighed and considered to have two mutually exclusive outcomes: presence or absence. When present, all RFs were parameterized with a value of RR modelled in R environment as Pert distributions with minimum values of 1, most likely values of 2 and maximum values of 3. These values translate into a minimum RR of one, a most-likely value of twice the risk of disease introduction for exposure to the RF and a maximum value of 3 times the risk to obtain a symmetrical Pert distribution. Where the RF was absent, RR was appointed a fixed value of 1.

2.3. Passive SSC

Passive surveillance is implemented for early detection of disease and can contribute to determining disease free status over time. Passive surveillance allows for the comprehensive coverage of the shrimp grow-out farm population, as all units are potentially subject to surveillance. The unit of analysis for the SePassiveSC are the shrimp grow-out farms.

2.3.1. Scenario tree

Detecting disease through passive surveillance relies on the probability that, at the farm level, infected animals show clinical signs of disease, that aquaculturists are aware of these signs and are motivated to report a suspected disease occurrence, that state veterinary authorities investigate the event and correctly suspect the occurrence of a specific viral disease and also on the diagnostic capacity of the tests to detect and confirm the presence of infection (Hadorn and Stark, 2008). The SePassiveSSC is determined according to Martin et al.,(2007). The scenario tree illustrating the detection process for the PassiveSC is illustrated in Fig. 3.

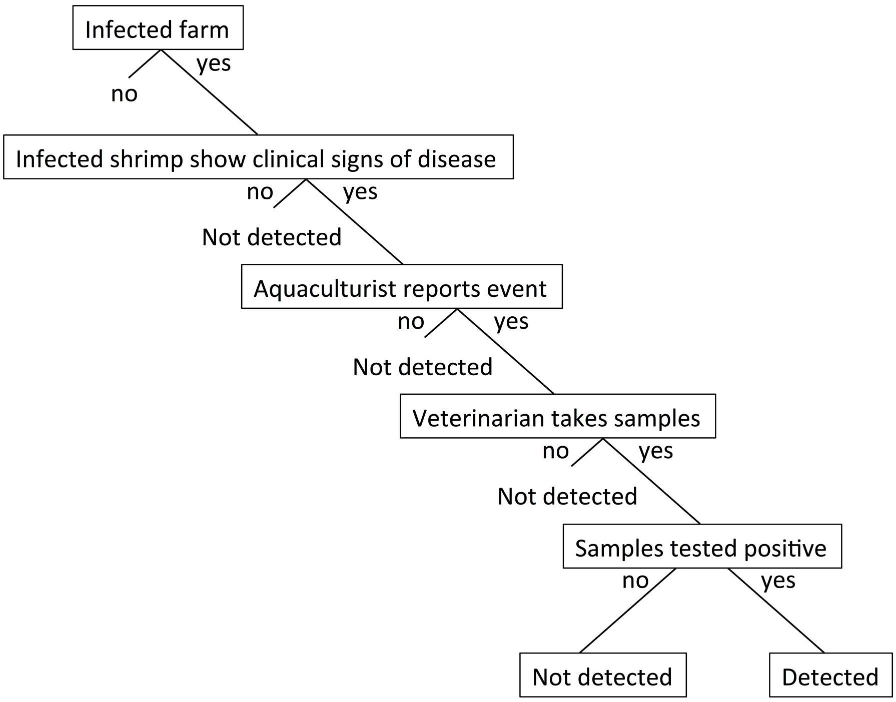

Figure 3: The scenario tree for the passive surveillance system component.

2.3.2. SePassiveSC

A simple approach was considered to calculate the SePassiveSC. The unit sensitivity for the PassiveSC (SeHP), that is, the probability that any randomly selected farm will give a positive test result, considers the probability that infection will result in shrimp developing clinical signs of disease (PRClinicalSigns), the probability of detection by aquaculturist (PrAquaculturist) and the probability of further investigation by a veterinarian (PrVeterinarian), along with the diagnostic test sensitivities (SeTests) and the number of $n_{passive}$ farms tested from the N farms in the population (Eq. 8) (E. Sergeant, personal communication, May 28, 2015). We consider that the shrimp sent for testing are infected and so the probability $P_{sampled\,fish\,infected}$ was set at 1. For the present example, we considered the PassiveSC would sample 10 farms $(n_{passive})$.

$$\begin{aligned} SeHP = Pr\,Clinical\,Signs\,*\,Pr\,Aquaculturist\,*\,Pr\,Veterinarian \\ (1-\,(1-\,P_{sampled\,fish\,infected}\,*\,SeTests)^{n_{passive}}) \end{aligned} \tag{8}$$

The SePassiveSC can then be determined through Eq.(9)(E. Sergeant, personal communication, May 28, 2015).

$$SePassiveSC \quad = \quad 1-(1-SeHP)^{(Ph^{*}*N)} \tag{9}$$

2.3.3. PassiveSC probabilities

The probabilities of the aquaculturist detecting symptoms of disease and contacting state veterinary authorities, and that state veterinary authorities suspect disease and conduct further investigation by sending samples for testing, can be considered for varying levels of disease awareness. These levels of varying awareness were parameterized according to Hadorn and Stark (2008) for three levels of low, medium and high disease awareness (Table 1). The level considered for analysis to conservatively represent the present level of awareness, is the lowest of these levels. Incrementing disease awareness may increase the SePassiveSC and can also be determined through the AquaVigil model.

The probability that animals show clinical signs of disease is difficult to determine for the listed notifiable viral shrimp diseases. Evidence suggests that long-term viral infections, such as

Table 1: Parameters for three levels of probability of disease awareness: low, medium and high.

	Pert distribution		
Probability of detection	Minimum	Most likely	Maximum
Low	0,1	0,2	0,3
Medium	0,4	0,5	0,6
High	0,7	0,8	0,9

WSSV, can be present without producing clinical signs of disease and disease outbreaks (Tsai et al., 1999). For this reason, the authors determined that a conservatively wide range of values should parameterize the Pert distribution for the probability of shrimp showing clinical signs of any listed viral disease, and so a minimum value of 1%, a most likely value of 5% and a maximum value of 10% were chosen.

2.4. Surveillance system sensitivity

The overall SSe of the AquaVigil model worked example for the state of Ceará was determined assuming the independence of the active and passive surveillance components (Eq.10) (Cameron, 2009).

$$SSe = 1 - (1 - SeActiveSSC) * (1 - SePassiveSSC) \tag{10}$$

2.5. Probability of freedom

The probability of freedom can be determined at the end of a surveillance period, when evaluating the information gathered through the surveillance components. The method to determine the probability of freedom also followed Martin et al., (2007). Using Bayes' theorem, the probability of the state being free from disease depends on the value of SSe and the prior probability that disease is present before the surveillance efforts are undergone. The authors selected an initial prior for the probability of freedom parameterized as an uninformed prior with a value of 0.5, for a 50% probability of disease presence or absence, following the example from terrestrial animal surveillance systems for disease freedom by Martin, (2008) and More, et al., (2009). It is important to mention that the aforementioned value, chosen by the authors in the absence of surveillance data, can inevitably bias disease freedom conclusions (Gustafson et al., 2010).

The AquaVigil model outputs the number of campaigns needed to achieve a desired probability of freedom when the same surveillance sampling strategy is repeatedly applied for active and passive surveillance, over what may be many periods of time. The probability of freedom is determined at the end of every surveillance campaign, in most cases, increasing the probability that the disease will be absent at the beginning of the next surveillance time period (TP). Considering the SSe and prior probability of freedom for the time period $Prior_{TP}$, a posterior value of probability of freedom (PostPrFree) is determined from Eq. (11) (Martin et al., 2007).

$$PostPrFree_{TP} = (1 - Prior_{TP})/(1 - Prior_{TP} * SSe_{TP}) \tag{11}$$

There is a need to consider that, during a time period, disease may be introduced into the state and so the value of PostPrFree is adjusted. In Brazil, import risk analysis minimizes the probability of disease entering the country (PrIntro), as does the significant geographic distance from other South American shrimp producing countries. In Ceará, the main route for disease introduction is through the uncontrolled movement of crustaceans and through the oceanic currents from neighbouring states. To account for the possibility of introduction of disease, the PrIntro was parameterized by the authors as a Pert distribution of minimum 1%, most likely 1,5% and maximum 2%, for a conservatively high estimate when compared to the values used for land-animal production systems, such as the point estimate in Frossling et al., (2009) of 0,833%. The value of PostPrFree$_{adjusted}$ is determined from the value of posterior probability of infection (PostPrInf$_{adjusted}$) (Eq.12) in Eq.(13) (Cameron, 2009).

$$PostPrInf_{adjusted} = PostPrInf + PrIntro - PostPrInf * PrIntro \tag{12}$$

$$PostPrFree_{adjusted} = 1 - PostPrInf_{adjusted} \tag{13}$$

2.6. Relationship between variables

The model output included a sensitivity analysis between RFs and the SeActiveSC and between the probabilities considered for the passive component and the value of SePassiveSC. Spearman rank-order correlation coefficients can identify which RFs and probabilities are most correlated to the values of both SeActiveSC and SePassiveSC. The model returns the values of the Spearman's correlation coefficient as a tornado plot. Values close to -1 and 1 indicate that the variables are highly negatively and positively correlated, respectively. The Spearman's correlation coefficients are determined and tested for statistical significance by constructing a test statistic t, where statistically significant Spearman rank-order correlations have an associated p-value of less than 5%. Correlation coefficients and significance test are determined through the Hmisc package (Harrell Jr., F.E. 2015).

3. Results and Discussion

3.1. Best sampling strategy for high-risk farm sampling

The sampling strategy for RBS considered the exclusive targeted sampling of high-risk grow-out farms, and therefore, farms with all four RFs. From the 325 grow-out farms in Ceará, 50 farms are characterized by the presence of all four RFs. Lower numbers of farms were sampled from then on, for 45, 40, 35, 30, 25 and 20 high-risk farms, to identify a more practical and cost-effective surveillance strategy. The number of animals to sample from each farm for representative random sampling is 32 and this was the value chosen for analysis.

The PassiveSC can contribute to increase SSe and influence the total number of campaigns needed to achieve the desired probability of freedom. Therefore, we can determine how many sampling campaigns and animals are needed for sampling with or without the contribution from this type of surveillance activity. Furthermore, we can consider the contribution from this surveillance component when awareness is high among farmers and veterinarians. Therefore, the number of campaigns, animals sampled and the surveillance system performance can be determined for three scenarios:

- Scenario 1: implementing only the ActiveSC.
- Scenario 2: implementing the ActiveSC and the PassiveSC with low disease awareness.
- Scenario 3: implementing the ActiveSC and the PassiveSC with high disease awareness.

3.1.1. Scenario 1

For scenario 1, the best sampling strategy for targeted sampling is that where 25 high-risk farms are sampled, for a total of 800 sampled animals and a single surveillance campaign (Table

2). Random and representative two-stage sampling of Ceará's 325 farms, would require 1856 animals to be sampled from 58 farms, sampling 32 animals per farm, to declare disease free status with a single surveillance campaign. Applying the previous targeted sampling strategy would account for a reduction of 56,9% in number of farms and of animals needed for testing to declare freedom from any one of the listed notifiable viral diseases. Furthermore, if pooled sampling of 5 animals were considered, the overall number of diagnostic tests needed to declare freedom would fall from 800 to 160. However, if pooling of samples were to be done, as is frequent in aquatic animal diagnostics, PCR test performance could be affected and values for test sensitivity and specificity reconsidered. Examples of surveys to determine disease freedom consider that the sensitivity of pooled testing is high, surpassing the value here chosen (Cannon et al., 1999;Lyngstad et al., 2010; OIE, 2014a).

Table 2: Scenario 1 sampling strategies to reach a probability of freedom superior or equal to 94,5% for the state of Ceará

Sampling Frame		Required sampling	
High-risk farms	Animals per farm	Campaigns	Total samples
50	32	1	1600
45	32	1	1440
40	32	1	1280
35	32	1	1120
30	32	1	960
25	32	1	800
20	32	2	1280

If the surveillance strategy were to privilege a lower sample size per campaign and active surveillance for a two-year period, the best strategy is the sampling of 20 high-risk farms, for a total of 1280 samples, collecting 640 animals per campaign.

3.1.2. Scenario 2

We can consider the contribution of the SePassiveSC to determine the number of campaigns and samples needed to determine disease free status. Here we conservatively estimated that 10 farms could have samples submitted for diagnostic exams through the PassiveSC. We verified that, for scenario 2, where the level of awareness is low among the farmers and veterinarians, the PassiveSC does not contribute to increase the value of the SSe to the point of changing the number of samples or campaigns needed to establish disease free status. The sampling frame and number of campaigns to determine disease free status is the same as that presented for scenario 1 and so is the best sampling strategy. The values for SeActiveSC and SePassiveSC, overall SSe and SR for Scenario 2 are summarized in Table 3.

Table 3: Scenario 2 surveillance system component sensitivities, overall SSes and SRs for surveillance sampling frames analysed for the state of Ceará, with corresponding 2,5%tiles

Sampling frame		Surveillance system performance			
High-risk farms	Animals per farm	SeActiveSC	SePassiveSC	SSe	SR
50	32	100,0%	3,10%	100,0%	1,06
		(100,0%;100,0%)	(1,10%;6,50%)	(100,0%;100,0%)	
45	32	100,0%	3,10%	100,0%	1,08
		(100,0%;100,0%)	(1,10%;6,50%)	(100,0%;100,0%)	
40	32	99,94%	3,10%	99,94%	1,10
		(99,66%;99,97%)	(1,10%;6,50%)	(99,67%;99,99%)	
35	32	99,66%	3,10%	99,67%	1,15
		(98,73%;99,89%)	(1,10%;6,50%)	(98,77%;99,90%)	
30	32	98,76%	3,10%	98,80%	1,22
		(96,55%;99,49%)	(1,10%;6,50%)	(96,65%;99,51%)	
25	32	96,50%	3,10%	96,62%	1,30
		(92,34%;98,22%)	(1,10%;6,50%)	(92,57%;98,28%)	
20	32	91,69%	3,10%	91,96%	1,37
		(85,13%;94,95%)	(1,10%;6,50%)	(85,57%;95,13%)	

3.1.3. Scenario 3

For scenario 3 we consider the increase of the level of disease awareness. There is an increase in SePassive contributing to the increase in overall SSe (Table 4). However, the increase in overall SSe does not translate into a change in number of samples required to determine freedom from disease in comparison to the previous scenarios.

Table 4: Scenario 3 surveillance system component sensitivities, overall SSes and SRs for surveillance sampling frames analysed for the state of Ceará, with corresponding 2,5%tiles

Sampling frame		Surveillance system performance			
High-risk farms	Animals per farm	SeActiveSC	SePassiveSC	SSe	SR
50	32	100,0% (100,0%;100,0%)	41.64% (19,26%;60,28%)	100,0% (100,0%;100,0%)	1,06
45	32	100,0% (100,0%;100,0%)	41.64% (19,26%;60,28%)	100,0% (99,97%;100,0%)	1,08
40	32	99,94% (99,66%;99,97%)	41.64% (19,26%;60,28%)	99,97% (99,79%;99,99%)	1,10
35	32	99,66% (98,73%;99,89%)	41.64% (19,26%;60,28%)	99,81% (99,20%;99,94%)	1,15
30	32	98,76% (96,55%;99,49%)	41.64% (19,26%;60,28%)	99,29% (97,82%;99,73%)	1,22
25	32	96,50% (92,34%;98,22%)	41.64% (19,26%;60,28%)	97,97% (95,08%;99,09%)	1,30
20	32	91,69% (85,13%;94,95%)	41,64% (19,26%;60,28%)	95,17% (90,22%;97,51%)	1,37

3.2. SR and relationship between variables

All SR values are above one, indicating a gain of sensitivity when performing RBS compared to an equivalent random sampling.

The AquaVigil model output included tornado plots of Spearman correlation coefficients for sensitivity analysis that identified the inputs most correlated to the values of SePassiveSC and SeActiveSC. For the chosen surveillance strategy rendering the smallest sample size and low awareness, the RF with the greatest correlation relationship to the value of SeActiveSC was RF1, the aquaculture grow-out farms with cultivating areas above 10 ha. The sensitivity analysis, given the same surveillance scenario, for the SePassiveSC, identified the probability of infected animals showing clinical signs of disease as the input variable with the greatest correlation relationship for this value (Fig. 4).

4. Conclusion

The prospective evaluation of different surveillance strategies allowed the identification of the best strategy to declare freedom from any listed notifiable viral disease affecting shrimp aquacul-

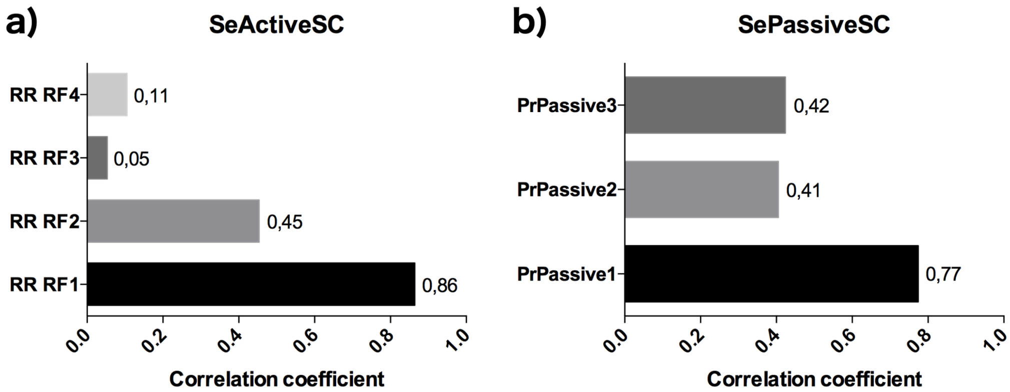

Figure 4: Tornado plots of Spearman correlation coefficients to identify the variables most correlated with the values of SeActiveSC (a) and SePassiveSC (b).

ture grow-out farms for the state of Ceará. The AquaVigil model took into account four RFs, among a wide range of other model parameters, returning the surveillance system performance before its application. The arbitrarity behind the choice of important model parameters inevitably alters the accuracy of the model. However, through their conservative estimation, the model can make disease freedom declaration a reality in the present context. Furthermore, it is important to state that the parameters can be changed as seen fit to evaluate different surveillance scenarios, as changes in any future context can occur and specific information made available. This model provides decision makers with a tool for strategical planning of future surveillance activities capable of early detection of disease and, in the event of sampling efforts failing to detect disease, to declare disease freedom. The model can also be applied to evaluate real data from the surveillance system once implemented, when a single sampling campaign is deemed sufficient to determine disease-free status or when the sampling protocol is repeated for consecutive surveillance campaigns. The AquaVigil model can evaluate other surveillance strategies for varying production systems, diseases and surveillance scenarios, provided they follow a similar surveillance outline.

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Conflict of interest statement

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Hierarchical clustering and partitioning to characterize shrimp grow-out farms in northeast Brazil

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Abstract

Shrimp farming in northeast Brazil is an important sector of the regional economies, contributing as a source of revenue and poverty alleviation. In recent years, shrimp aquaculture has faced many challenges, ranging from the occurrence of viral diseases to changes in market access. Considering the past and present challenges faced by the shrimp farmers in northeast Brazil there is a current need to identify the present trends in shrimp farming when striving for the sustainable development of this important sector os the Brazilian economy. To this aim, the exploratory data analysis methods of multiple correspondence analysis and hierarchical and partitional clustering were applied to the data collected through a national census to extract the greatest amount of information and describe present trends in shrimp farms, identifying gaps and needs. The results of the analysis will contribute to improve management practices and policy-making for sustainable shrimp farming in northeast Brazil.

Keywords: Shrimp aquaculture northeast Brazil; hierarchical clustering and partitioning.

1. Introduction

The farming of Pacific white shrimp *Litopennaeus vannamei* in northeast Brazil has been an important source of income for large-scale producers and for the rural community small-scale farmers. However, the farming of Pacific white shrimp in Brazil has been faced with many challenges. Generally accepted issues are identified as compromising to the sectors sustainability. These include the suitability of management practices, biosecurity measures and the occurrence of diseases (ABCC/MPA, 2013). The understanding of the present trends in shrimp farming in

Brazil, with specific focus on management and the farmer's perspective on shrimp farming, is fundamental to assure the sectors sustainable development. To this aim, the exploratory data analysis methods of multiple correspondence analysis (MCA) and hierarchical and partitional clustering were applied to secondary data generated from a census of shrimp grow-out farms in northeast Brazil, to characterize these farms by studying the resemblances among them with respect to different variables. The principal component method of MCA was applied as a pre-processing step to denoise the data set for stable hierarchical and partitional clustering, while balancing the influence of the many variables considered (Husson et al., 2011). The information from there derived, can be used by policy makers and other stakeholders for knowledge-based policy development and farm management.

2. Methodology

2.1. Data source

A survey to characterize the shrimp aquaculture sector's productivity, technological, economical and socio-environmental aspects was commissioned by the Ministry of Aquaculture and Fisheries (MPA) and carried out by the Association of Brazilian Shrimp Aquaculturists (ABCC) for 2011 (ABCC/MPA, 2013). The variables collected through the ABCC/MPA census were determined by the ABCC and their collaborators and a descriptive data analysis performed on a set of these variables is presented in the publication "*Levantamento da Infraestrutura Produtiva e dos Aspectos Tecnológicos, Econômicos, Sociais e Ambientais da Carcinicultura Marinha no Brasil em 2011*" (ABCC/MPA, 2013). The data collected from the aforementioned census was the data used for the present analysis. Moreover, the aforementioned publication did not perform any of the data analysis methods here described and also neglected to make reference to a number of categorical variables retrieved from the census. Thus, 22 additional categorical variables were included in the present analysis.

The surveyed shrimp grow-out farms for the census included those located in the States that make up the northeast coast-line of Brazil (Figure 1): the states of Pará (PA), Maranhão (MA), Piauí (PI), Ceará (CE), Rio Grande do Norte (RN), Paraíba (PB), Pernambuco (PB), Alagoas (AL), Sergipe (SE) and Bahia (BA). From the 1222 shrimp grow-out farms in these states, 1179 were included in the present analysis as they represent the farms with the usable data collected through the census (ABCC/MPA, 2013). The 36 categorical variables included in the census and chosen for the present analysis are presented in Table 1. These variables will be analysed to identify trends in shrimp grow-out farming. An initial approach will consider all variables as active in the construction of the principal components. To obtain a better understanding of the productivity and management practices, as well as the shrimp farmers concerns, their awareness of what constituted a biosecurity measure and which of these were desirable to be applied at their farm, two groups of categorical variables were considered to characterize the establishments. In order to create these sets of variables, the complete data set was divided into two groups variables of variables: Group A and Group B (Table 1).

Figure 1: Map of the States where shrimp grow-out farms were surveyed in northeast Brazil.

Table 1: Variables analysed for characterizing shrimp grow-out farms

Group A	
Variables	Categories
1. Grow-out farm in terms of production area	1- Micro producer farmed area <5 ha 2- Small producer farmed area]5-10]ha 3- Medium producer farmed area]10-15]ha 4- Large producer farmed area>50 ha
2. Shrimp production (tons) compared to the national average of 3,51 tons/ha/year	1- Production above national average 2- Production bellow national average
3. Stocking density	1- Low density<10 shrimp/m^2 2- Medium density]10-30] shrimp/m^2 3- High density]30-50] shrimp/m^2 4- Very high density >50 shrimp/m^2
4. Number of production cycles per year	1- 1-2 cycles/year 2- 3 cycles/year 3- ≥4 cycles/year
5. Environmental license	1-Yes 2- No
6. Lowest shrimp weight at harvest (grams)	1- < 7 grams 2-]7-10] grams 3-]10-12] grams 4-]12-15] grams 5- >15 grams
7. Mechanical aeration of ponds	1- Yes 2- No
8. Nursery on site	1- Yes 2- No
9. Fenced grow-out pond area for post-larva PL reception	1- Yes 2- No
10. Receive technical assistance	1- Yes 2- No
11. Type of soil treatments applied, from liming, chlorination, exposure to sunlight, ploughing and use of probiotics	1- One treatment 2- Two treatments 3- Three treatments 4- Four treatments 5- Five treatments
12. Use feeding trays	1- Yes 2- No
13. Number of controlled water quality variables, from oxygen, salinity, temperature, turbidity and nitrogen	1- One variable 2- Two variables 3- Three variables 4- Four variables 5- Five variables
14. Use of any kind of probiotic	1- Yes 2- No
15. Practice polyculture	1- Yes 2- No
16. Destination of shrimp for processing	1- Refrigerate 2- Frozen 3- Smoked or toasted
17. Main source of water	1- Estuary 2- Ocean 3- River

Continued on next page

Table 1: Variables analysed for characterizing shrimp grow-out farms

Group A	
Variables	Categories
	4- Well
18. Testing for disease	1- Wet mount 2- No testing 3- PCR or histopathology 4- Both
19. Receive outside support for diagnostics	1- Yes 2- No
20. Have sedimentation tanks on site	1- Yes 2- No

Group B	
Variables	Categories
21. Satisfied with post-larvae (PL) quality	1- Yes 2- No
22. Satisfied with quality of feed	1- Yes 2- No
23. Satisfied with quality of antibiotics and fertilizers	1- Yes 2- No
24. How farmers regard producing in the presence of disease	1- No opinion 2- Optimistic 3- with reservations 4- Pessimistic
25. Concerned with implementing new technologies	1- Yes 2- No
26. Part of an aquaculture organization	1- Yes 2- No
27. Looking to increase production	1- Yes 2- No
28. Farmers claim awareness of what make up biosecurity measures	1- Yes 2- No
29. Would use specific disease free PL (SPF)	1- Yes 2- No
30. Would use specific disease resistant PL (SPR)	1- Yes 2- No
31. Would use foot bath	1- Yes 2- No
32. Would use wheel dip	1- Yes 2- No
33. Would implement hygiene practices for personnel	1- Yes 2- No
34. Would use sanitation of equipment	1- Yes 2- No
35. Would apply animal control	1- Yes 2- No
36. Would apply farm fencing	1- Yes 2- No
	Concluded

2.2. Data analysis

Multiple correspondence analysis is a simple correspondence analysis carried out on an indicator matrix, with grow-out farms as rows and categorical variables as columns. Multiple correspondence analysis (MCA) was performed on all variables and for each of the groups of categorical variables as a denoising method to separate the signal on the first dimensions from the noise on the last dimensions (Husson, F., et al., 2010). After performing the data denoising, hierarchical clustering and partitioning for better data visualization is applied on the first dimensions, extracting the essential of the information from the original data. The MCA and HCPC (hierarchical clustering on principle components) functions used in the analysis were implemented in R environment, through the FactoMineR package (R Development Core Team, 2008). The R script that performed the present analysis is made available as Supplementary Document 1, with step-by-step detailed information on the chosen package functions and parameters. The data set of farms and categorical variables is also provided as Supplementary Document 2, to simulate the results here obtained.

2.2.1. Missing values

The missing values in the data set made up a total of approximately 21% of the entries. The missMDA package allows the imputation of missing values in the data sets. The imputed values are excluded from the construction of the principal components as they are treated as an additional level in the MCA analysis. Given the high amount of missing values, the regularised iterative MCA algorithm is applied to avoid over fitting. Here, an important step consists in determining a value for the number of dimensions in the reconstruction formula to impute the disjunctive matrix used in MCA. This value was set as the smallest value corresponding to the greatest transition between the mean square error of prediction for each dimension tested (Husson, F. & Josse, J., 2015). Categorical variables not described in the original censos, due to the existence of missing values, can know be analised and used to characterize the shrimp grow-out farms.

As partitional clustering was performed for the whole data set and then for the data in Group A and Group B, the treatment of missing values was repeated considering the whole data set and then for the data considered for the two groups. The estimated number of dimensions to impute the missing values was determined using a regularised iterative MCA algorithm (Husson F.& Josse J. 2015). For any of the three strategies, the number of components chosen to predict the missing entries was determined as 1.

2.2.2. MCA

MCA was the principal component method chosen to perform preprocessing for partitional clustering. Our initial data set accounted for 1179 shrimp grow-out farms, with 20 variables in Group A, 17 variables in Group B and a total of 36 categorical variables in all. The principal aim of MCA was to describe the large data sets here analysed, using fewer, uncorrelated variables while retaining the greatest amount of information possible. The first components accounting for as much of the variability in the data as possible, summarizing the greatest amount of information from the data set, are called from the MCA analysis to perform partitional and hierarchical clustering (François, H., Josse, J. & Pagès, J., 2010). Therefore, the MCA was used as a method of factorial analysis for denoising to perform hierarchical and partitional classification on the components that described a chosen value of roughly 70% of the data.

MCA can be carried out on the Burt table, the inner product of the indicator matrix of grow-out farms and categorical variables, as is usually done in MCA. The inertias associated with the first components for MCA of the Burt table are higher than the inertia associated with MCA on the indicator matrix (Husson et al., 2011). As a large number of grow-out farms will be considered and the Burt table contains the information as associations between categories, the Burt table was the preferred data storage method for the correspondence analysis here performed on the chosen active variables.

MCA was performed on the 36 categorical variables and then considering the categorical variables divided into Group A and in Group B as active in the construction of the components. When all variables are considered, 8 components described 71% of the data. For Group A, 9 components describe 72% of the data set and for Group B 5 components describe 75% of the data set. A second MCA is performed considering only these principal components for further analysis.

2.2.3. Partitional clustering

The three methods of MCA, hierarchical clustering and partitional clustering are combined with the general aim of constructing clusters of grow-out farms from the denoised data sets, to describe the clustered farms in terms of their characteristics. Agglomerative hierarchical clustering and partitioning was performed through the HCPC function on the results of the MCA outputs, according to the methodology by Husson, F., Lê, S. and Pagès, J. (2010, 2011). The HCPC function makes use of Euclidean distances to define the distance between individuals and the Ward's agglomeration method to construct the hierarchical tree. A hierarchical tree is a sequence of nested partitions, from the individual as a class to a more general partition that includes all individuals. Each branch groups individuals with a shared set of properties. Apart from the similarity among them, individuals in a group differ from other grouped individuals. The Ward's agglomeration method consists in regrouping individuals by maximizing the quality of the partitioning. Huygen's theorem provides the framework for combining clusters so that the growth of within cluster-inertia is minimum at each step of the algorithm, in order to construct homogeneous clusters. The hierarchy is represented by a dendrogram illustrating the gain of within-cluster inertia when we partition Q clusters to Q-1 clusters. The individuals are also arranged in the hierarchical tree according to the first principal component as far as possible (Husson, F., et al, 2010; Husson, F., Lê, S. & Pagès, J., 2011).

The number of clusters can then be chosen visually by looking at the overall shape of the tree or at a level suggested by the bar plot of the gain in within inertia when we go from one cluster to two clusters for the fisrt bar, from two clusters to three clusters for the second bar, and so on (Husson F., et al. 2015). The ideal level is suggested by the HCPC function. We construct the hierarchical tree and now consolidate the results using the k-means method for partitional clustering with 10 maximum iterations of this algorithm. The partition obtained from the cut of the hierarchical tree is introduced as the initial partition for the k-means algorithm (Husson, F., Josse, J. & Pagès, J., 2010).

The categories of the cluster variable are characterised by the categories of the categorical variables (Husson, F., Josse, J. & Pagès J. 2010). To evaluate the relationship between them, one must compare their proportions through a statistical test based on the hypergeometric distribution. The hypothesis test based on the hypergeometric distribution states the null hypothesis that the two proportions should be equal (Lê & Worch, 2015). Categories of the categorical variables significantly linked to the cluster are identified in ascending order of p-value and for each category of the categorical variable, and the v-test (test-value), the quantile of a normal distribution associated to the p-values, is determined (Husson, F., Lê, S. & Pagès, J. 2011). A value of the v-test greater than 1.96 corresponds to a p-value less than 0.05 (Husson, F., Josse, J. & Pagès J. 2010). The over-represented categories of all the categorical variables are organised from most to least characteristic, when the v-test is positive (Husson, F., Lê, S., Pagès, J. 2011). Under-represented categories, with negative v-test values, are omitted from the results. Only the over-represented categories characterizing the cluster will be described, to ease interpretation.

3. Results and Discussion

The results from the clustering performed on the outputs of the MCA are presented by the dendrograms in Figure 2. For all active variables and for variables in Group A, the number of clusters suggested resulted in the hierarchical tree being cut into three clusters. For variables in Group B, the hierarchical tree was cut at a level where the inertia gain when clustering two clusters was greater than that corresponding to cutting the tree at a lower level.

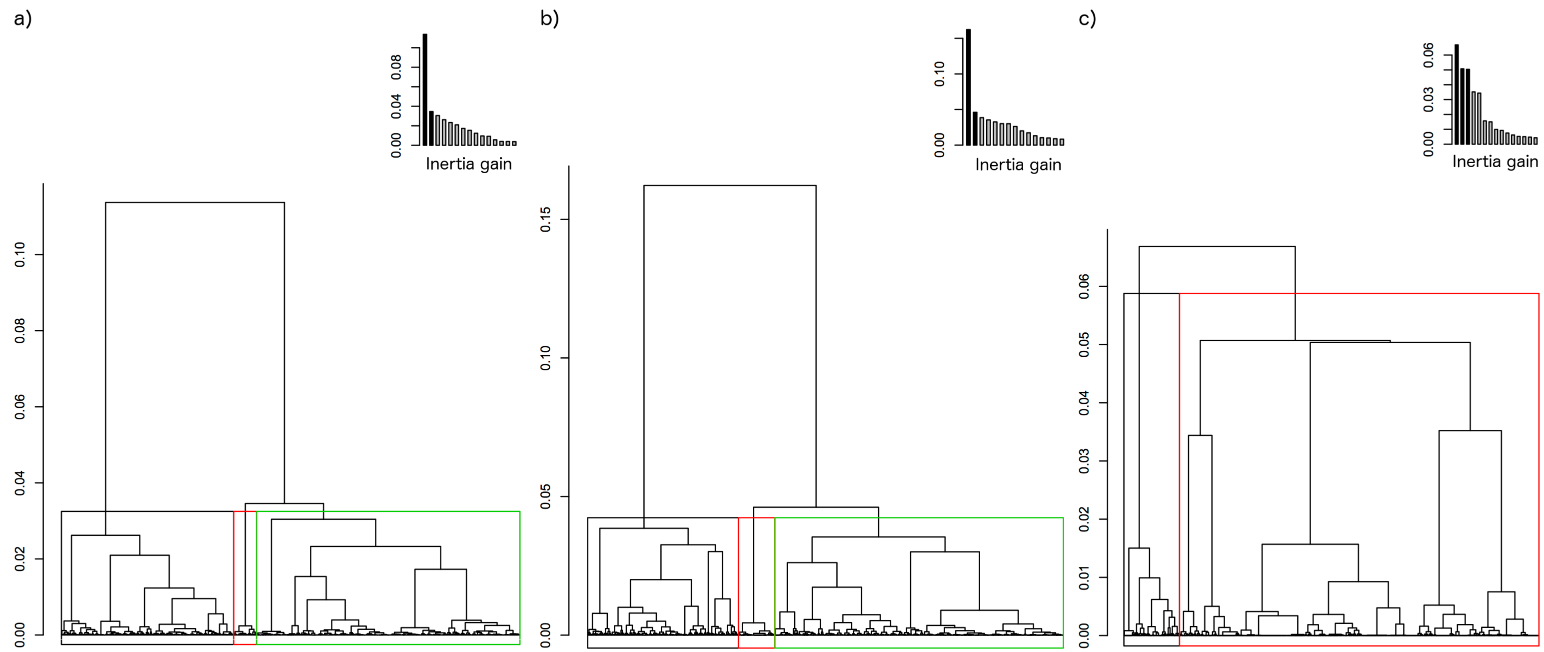

Figure 2: Map of the States where shrimp grow-out farms were surveyed in northeast Brazil.

3.1. Cluster description considering all variables

Cluster description considering all variables is summarized in Table 2. The first cluster constructed considering all variables is most characterized by farmers producing above the national average, with an environmental license, that make use of probiotics and claim awareness of the biosecurity measures. To a lesser extent, this cluster is also characterized be farms that are medium and large scale and that enjoy a more technified level of production, employing the use of mechanical aeration, nursery on site, receive technical assistance, use feeding trays, apply numerous soil treatments, control many water variables, use sedimentation tanks, among other indicators of high investment capacity. The contribution of the variable "Satisfied with the quality of antibiotics and fertilizers" indicates access to and acquisition capacity of quality antibiotics and fertilizers to the farm. Many farms receive technical support for diagnostics and use wet-mount techniques to diagnose disease in their shrimp, which could be a shortcoming, as this technique can fail to detect important infectious agents. Farmers are concerned with implementing new technologies, demonstrating willingness to further invest in their farms, further supported by their claim of "Looking to increase production". Additionally, the large weight of shrimp harvested and ability to freeze their shrimp makes for easier, planned market access. They consider the most important biosecurity practices the hygiene of personnel, the sanitation of equipment, the control of animal movement onto the farm and the application of fencing to the farm perimeter, and to a lesser extent the use of SPF, SPR, wheel dip and foot bath.

The categories that best represent cluster 2 allow us to identify this cluster as characterized by farmers who make use of PCR or histopathology to identify infection at the farm level, indicating awareness of the importance of detecting animals with subclinical infection. Contrary to what may be expected, the farmers in this cluster do not consider as important the implementing of many of the biosecurity variables, such as the use of SPR and the hygiene applied to personnel. They are concerned with the application of new technologies and produce without environmental license, which could difficult access to financial aid. An indicator of the low investment capacity could be the absence of mechanical aeration, the production bellow national average and the micro-scale of the farms. The third cluster considering all variables is most characterized by farmers who are micro-scale, do not have an environmental license, do not make use of probiotics, do not test for the presence of disease, do not use sedimentation tanks and claim they are unaware of what are considered biosecurity practices. Production levels are bellow average and low-technified production is present, given the absence of mechanical aeration, nurseries on site and the control of water variables. In spite of their limitations and low production, farmers are not concerned with implementing new technologies which could derive from the knowledge of the limitations to increase current production levels. Further vulnerability to the sustainable production of shrimp in this cluster is suggested by the absence of technical support for general production and for disease diagnostics and the optimism regarding production in the presence of disease. Differences between clusters 2 and 3 allow us to observe two groups of micro-scale farmers, with different ambitions when looking to increase production, implement new technologies and testing for disease presence. Furthermore, the variables characterizing farms in cluster 1 oppose those characterizing clusters 2 and 3, indicating a strong polarization in responses between these farms.

Table 2: Description of the clusters for all variables, by variable categories significantly linked to the cluster (p-value< 0,05) where values emphasised by the asterisks (***) denote a v-test value $\geq$ 20, (**) a v-test value]20-10], (*) a v-test value]10-5] and no asterisk a v-test value]5-0].

Variables	Cluster 1	Cluster 2	Cluster 3
1. Grow-out farm in terms of production area	Medium**; Large**;Small*	Micro	Micro***
2. Shrimp production compared to the national average	Above ***	Bellow	Bellow**
3. Stocking density	High*; Very high*	Low	Medium*
4. Number of production cycles per year	1-2*	3	$\geq$ 4
5. Environmental license	Yes***	No*	No***
6. Lowest shrimp weight at harvest (grams)	]12-15]*; >15*	<7	]7-10]*
7. Mechanical aeration of ponds	Yes**	No*	No**
8. Nursery on site	Yes**	No	No**
9. Fenced grow-out pond area for post-larva PL reception	Yes*	No	
10. Receive technical assistance	Yes**	No	No**
11. Number of soil treatments applied	5**;4**	1	2*;1
12. Use feeding			

Continued on next page

Table 2: Description of the clusters for all variables, by variable categories significantly linked to the cluster (p-value< 0,05) where values emphasised by the asterisks (***) denote a v-test value ≥ 20, (**) a v-test value]20-10], (*) a v-test value]10-5] and no asterisk a v-test value]5-0].

Variables	Cluster 1	Cluster 2	Cluster 3
trays	Yes**	Yes	No**
13. Number of controlled water quality variables	3**; 4**;2*;1;5		
14. Use probiotics	Yes***		No***
15. Practice polyculture	Yes		No
16. Destination of shrimp for processing	Frozen*; Smoke/Toast		Refrigerate*
17. Main source water	River; Well	Estuary*	Estuary
18. Testing for disease	Wet mount**; Both*	PCR/Histo.**	No***
19. Receive outside support for diagnostics	Yes**		No**
20. Have sedimentation tanks	Yes**		No***
21. Satisfied with PL quality			
22. Satisfied feed quality	Yes	Yes	
23. Satisfied with quality of antibiotics and fertilizers	Yes**	No	No*
24. How farmers regard producing in the presence of disease	Reservations; Pessimistic	Optimistic; No opinion	Optimistic*
25. Concerned with implementing new technologies	Yes**	Yes*	No**
26. Part of an aquaculture organization	Yes		No
27. Looking to increase production	Yes	Yes	No
28. Farmers claim awareness of biosecurity measures	Yes***		No***
29. Would use specific disease free PL (SPF)	Yes*; No		
30. Would use specific disease resistant PL (SPR)	Yes	No**	
31. Would use foot bath	Yes*; No		
32. Would use wheel dip	Yes*; No		
33. Would implement hygiene practices for personnel	Yes**	No*;Yes*	
34. Would use sanitation of equipment	Yes**	No**	
35. Would apply animal control	Yes**		
36. Would apply farm fencing	Yes**		
			Concluded

3.2. *Cluster description for Group A*

For greater understanding of the management practices at the farm level, a second MCA analysis considered active variables those related to management practices. Cluster description considering Group A variables is summarized in Table 3.

The first cluster identified was most characterized by farms that produce above the national average, with environmental license, making use of probiotics. The remaining variables that characterized the cluster do so in the same way as indicated in cluster 1 for all variables.

The grouping of management variables in Group A was useful in analysing the differences between two groups of farmers, also identified when analysing all variables as clusters 2 and 3. For this second cluster, the variables that most characterize the cluster are the micro-scale of farms, the production bellow the national average, the low stocking density, absence of environmental license, largest weight of]10-12] grams at time of harvest, no mechanical aeration nor feeding trays, the application of 2 soil treatments, the absence of use of probiotics, the absence of testing for disease and the main source of water from rivers.

A third cluster was identified where categories were very similar to the aforementioned cluster 2. This cluster differs from the previous cluster mainly as it is characterized by farmers that make use of medium stocking densities with a smaller weight for the shrimp at time of harvest, making use of feeding trays, water from an estuary and performing either no testing or performing sensitive methods for disease detection such as PCR or histopathology.

Table 3: Description of the clusters for Group A, by variable categories significantly linked to the cluster (p-value$<$ 0,05) where values emphasised by the asterisks (***) denote a v-test value $\geq$ 20, (**) a v-test value]20-10], (*) a v-test value]10-5] and no asterisk a v-test value]5-0].

Variables	Cluster 1	Cluster 2	Cluster 3
1. Grow-out farm in terms of production area	Medium**; Large**;Small	Micro*	Micro**
2. Shrimp production compared to national average	Above***	Bellow**	Bellow*
3. Stocking density	High*; Very high*	Low**	Medium*
4. Number of production cycles per year	1-2*	$\geq$4	
5. Environmental license	Yes***	No**	No**
6. Lowest shrimp weight at harvest (grams)	]12-15]*;$>$15*	]10-12]**	]7-10]*;$<$7*

Continued on next page

Table 3: Description of the clusters for Group A, by variable categories significantly linked to the cluster (p-value< 0,05) where values emphasised by the asterisks (***) denote a v-test value $\geq$ 20, (**) a v-test value]20-10], (*) a v-test value]10-5] and no asterisk a v-test value]5-0].

Variables	Cluster 1	Cluster 2	Cluster 3
7. Mechanical aeration of ponds	Yes**	No**	No*
8. Nursery on site	Yes**	No*	No**
9. Fenced grow-out pond area for PL reception	Yes*	No	
10. Receive technical assistance	Yes**	No*	No*
11. Number of soil treatments applied	5**; 4*	2**	1*;3
12. Use feeding trays	Yes*	No**	Yes*
13. Number of controlled water quality variables	3**; 4**;2*;5; 1		
14. Use of probiotics	Yes***	No**	No**
15. Practice polyculture	Yes		No
16. Destination of shrimp for processing	Frozen*; Smoke/Toast	Refrigerate	Refrigerate
17. Main source of water	River; Well; Ocean	River**	Estuary**
18. Testing for disease	Wet mount**; Both*	None**	None**;PCR/Histo.*
19. Receive outside support for diagnostics	Yes**	No*	No*
20. Sedimentation tanks on site	Yes**	No*	No**

3.3. Cluster description for Group B

For Group B, variables that describe the farmers general views and concerns, their view on the future of shrimp farming in the presence of disease and the importance of biosecurity measures were grouped for analysis. Cluster description considering Group B variables is summarized in Table 4.

The cluster analysis identified two clusters of farms. The first cluster is characterized by farmers who are satisfied with PL quality, feed quality and quality of antibiotics and fertilizers, are optimistic in regards to producing in the presence of disease and are not part of an aquaculture organization. In opposition to this cluster are farmers who are unsatisfied with the quality of the PL supplied to their farms and the quality of feed, are pessimistic or have no opinion on the threat of producing in the presence of disease and are part of an aquaculture organization.

This clustered revealed a characterization of farms not yet identified when analysing all variables. Previously, the cluster 1 for all variables was characterized by farmers satisfied with the quality of the antibiotics, fertilizers and PL they used on their farms as the same group characterized by farmers pessimistic or with reservations on producing in the presence of disease and belonging to an aquaculture organization. The present cluster is now characterized by farmers that are satisfied with the general production inputs, are optimistic and do not belonging to an aquaculture organization. Similarly, cluster 2 of the present analysis also characterizes farms in a different way, much like the aforementioned results.

The influence between considering all variables or only those chosen for Group B clearly influenced the results. A cautious interpretation of the variables in Group B would lead us to consider the variables most significantly linked to the clusters. This suggests that the cluster characterized by more satisfied farmers is also that where farmers are more optimistic, opposed to a group of farmers clearly unsatisfied with the quality of the considered inputs.

Table 4: Description of the clusters for Group B, by variable categories significantly linked to the cluster (p-value$<$ 0,05) where values emphasised by the asterisks (***) denote a v-test value $\geq$ 20, (**) a v-test value]20-10], (*) a v-test value]10-5] and no asterisk a v-test value]5-0].

Variables	Cluster 1	Cluster 2
1. Satisfied with post-larvae (PL) quality	Yes***	No***
2. Satisfied with quality of feed	Yes**	No***
3. Satisfied with quality of antibiotics and fertilizers	Yes*	No*
4. How farmers regard producing in the presence of disease	Optimistic*	Pessimistic; No opinion
5. Part of an aquaculture organization	No	Yes

4. Conclusion

As was expected, an increasingly greater difference is observed between the micro-scale grow-out farms and the large-scale grow-out farms in northeast Brazil. There is a recognizable increase in technical capacity to produce more and healthier shrimp considering equipment, expertise and awareness where larger, higher production farms are concerned.

Smaller-scale farmers have different concerns and vulnerabilities expressed not only through their lower production and investment capacity but also their views on producing in the presence of disease and awareness of biosecurity measures. The more vulnerable, small-scale farms need to be informed of what are biosecurity practices, their advantages and the threat of producing in the presence of disease. Furthermore, these farmers should be motivated to take an active role in the industry and participate in aquaculture organizations. Additionally, their vulnerability increases from the absence of support for diagnostics and technical assistance that could derive from belonging to an organization of shrimp producers. Furthermore, there is a difference in the shrimp supplied from these producers, highlighting a difference in market access.

Two groups of farmers were identified as micro-scale farmers, producing different densities of shrimp, harvesting the shrimp at different weights and where one group performed highly sensitive diagnostic testing to detect disease. Therefore, this division between these initially similar types of farms highlits important differences in management and production.

When considering biosecurity and the farmers general views, two distinct groups of farmers illustrate two opposing groups: one where we can identify a group where there is a general satisfaction and optimism among farmers and to a less extent, the choice of not participating in aquaculture organizations, and another with an opposing stance on all the aforementioned variables. General information on biosecurity and disease awareness should be made available for all grow-out farmers and general motivation to participate actively in the industry by taking part in aquaculture organizations, implemented.

The trends and highlighted vulnerabilities identified in this study can be a valuable tool for all stakeholders involved in shrimp farming: policy makers can use this information to develop better strategies to secure the sector's sustainability and development; shrimp farmers are alerted to their constraints and weaknesses; and those providing technical assistance are alerted to where their expertise is most needed.

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Conflict of interest statement

The authors wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

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